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(54) Title: OLFACTORY AND PHEROMONES G-PROTEIN COUPLED RECEPTORS

(57) Abstract: The present invention is related to an olfactory or pheromone G-protein coupled receptor having an amino acid sequence which presents 95% sequence identity with the sequence SEQ ID NO. 1.

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OLFACTORY AND PHEROMONES G-PROTEIN COUPLED RECEPTORS

Field of the invention

10 [0001] The present invention is related to newly identified, isolated and purified members of the family of olfactory ligand (odorant or any other molecule able to interact with said receptor) and the family of pheromone ligand G-protein-coupled (preferably human) receptors as 15 well as to the various uses that can be made of said receptors.

[0002] The invention is also related to the polynucleotides sequences encoding said (preferably human) receptors.

20 [0003] The invention is further related to methods using receptor polypeptides and polynucleotides applicable to diagnostic and treatment in receptor-mediated disorders.

[0004] The invention is further related to ligand-screening methods using the receptor polypeptides and 25 polynucleotides, to identify agonists and antagonists used as improved flavours or perfumes, as well as the prevention and/or treatment of various disorders.

[0005] The invention further encompasses agonists and antagonists based on the said olfactory and pheromones 30 receptor polypeptides and polynucleotides and biosensors comprising said receptor polypeptides.

[0006] The invention is further related to procedures for producing the receptor polypeptides and

polynucleotides according to the invention, preferably by genetic recombinant methods.

Background of the invention

[0007] G-protein coupled receptors (GPCRs) are proteins responsible for transducing a signal within a cell. GPCRs have usually seven transmembrane domains. Upon binding of a ligand to an extra-cellular portion or fragment of a GPCR, a signal is transduced within the cell that results in a change in a biological or physiological property or behaviour of the cell. GPCRs, along with G-proteins and effectors (intracellular enzymes and membrane channels modulated by G-proteins), are the components of a modular signalling system that connects the state of intra-cellular second messengers to extra-cellular inputs.

[0008] GPCR genes and gene products are potential causative agents of disease and these receptors seem to be of critical importance to both the central nervous system and peripheral physiological processes.

[0009] The GPCR protein superfamily is represented in five families : Family I, receptors typified by rhodopsin and the beta2-adrenergic receptor and currently represented by over 200 unique members; Family II, the parathyroid hormone/calcitonin/secretin receptor family; Family III, the metabotropic glutamate receptor family, Family IV, the CAMP receptor family, important in the chemotaxis and development of *D. discoideum*; and Family V, the fungal mating pheromone receptor such as STE2.

[0010] G proteins represent a family of heterotrimeric proteins composed of α, β and γ subunits, that bind guanine nucleotides. These proteins are usually linked to cell surface receptors (receptors containing seven transmembrane domains).

[0011] Following ligand binding to the GPCR, a conformational change is transmitted to the G protein, which caused the α-subunit to exchange a bound GDP molecule for a GTP molecule and to dissociate from the βγ-subunits.

[0012] The GTP-bound form of the α, β and γ-subunits typically functions as an effector-modulating moiety, leading to the production of second messengers, such as cAMP (e.g. by activation of adenyl cyclase), diacylglycerol or inositol phosphates.

[0013] Greater than 20 different types of α-subunits are known in humans. These subunits associate with a small pool of β and γ subunits. Examples of mammalian G proteins include Gi, Go, Gq, Gs and Gt. G proteins are described extensively in Lodish et al., *Molecular Cell Biology*, (Scientific American Books Inc., New York, N.Y., 1995), the contents of which are incorporated herein by reference.

[0014] Known and unknown GPCRs constitute now major targets for drug action and development.

[0015] More than 300 GPCRs have been cloned thus far and it is generally assumed that it exists well over 1000 such receptors. Mechanistically, approximately 50-60% of all clinically relevant drugs act by modulating the functions of various GPCRs (Cudermann et al., *J. Mol. Med.*, Vol. 73, pages 51-63, 1995).

Summary of the invention

[0016] The present invention is related to newly isolated and purified identified members of olfactory (odorant/ligand) and pheromones/ligands (SEQ ID NO. 1 to SEQ.ID NO. 548) and G-protein-coupled receptors as well as to polynucleotide sequences including recombinant sequences encoding said receptors, described hereafter.

[0017] The present invention is also related to non-described nucleotide and/or amino acid sequences homologous to the sequences corresponding to the receptors described hereafter.

[0018] Homologous sequences mean sequences which present a high sequence identity (which present an identity higher than 75%, 80%, 85%, 90% or 95%) with the complete sequence described hereafter.

[0019] Another aspect of the present invention is related to a specific active portion of said sequences or a libraries of said active portion (ligand binding) of these sequences. Said portions could be partial or deleted receptors which comprise modifications (punctual mutations) or deletions upon the complete nucleotide or amino acid sequences and which still maintain the active site(s) necessary for the binding of a specific ligand able to interact with said receptors.

[0020] Homologous sequences of the sequences according to the invention may comprise similar receptors which exist in other animal (vertebrates, preferably mammalian) or specific human populations, but which are involved in the same biochemical pathway.

[0021] Such homologous sequences may comprise addition, deletion or substitution of one or more amino acids or nucleotides, which does not substantially alter the functional characteristics of the receptor(s) according to the invention in order to form preferably an hybrid polypeptide with another transmembrane protein, suitable for rapid ligand screening.

[0022] Thus, the invention encompasses also a receptor and corresponding nucleotide sequence having exactly the same amino acid or nucleotide sequences (derived from olfactory neurons or olfactory epithelium) as shown in the enclosed sequence listing, as well as

molecules which differ, but which are not retaining the basic qualitative binding properties of the receptors according to the invention.

[0023] The invention is preferably related to said human receptors characterised by the complete nucleotide and amino acid sequences described hereafter, agonist and antagonist compounds or inhibitors, preferably antibodies or specific hypervariable portions thereof that bind specifically to said receptors (i.e. that have at least a 10 fold greater affinity for said receptors than any other naturally occurring antibody) and specifically antibodies made by a process involving the injection of a pharmaceutically acceptable preparation of such amino acid sequence into a animal capable of producing antibodies directed against said receptor.

[0024] For instance, a monoclonal antibody to the receptor according to the invention is obtained by injecting of an expression plasmid comprising the DNA encoding said receptor into a non human mammal and than fusing mouse spleen cells with myeloma cells.

[0025] The present invention is also related to the polynucleotide according to the invention, possibly linked to other expression sequences and incorporated into a vector and host cells transformed by such vector (plasmid, virus such as an adenovirus, liposomes, cationic vesicles,...).

[0026] The present invention is also related to the recombinant, preferably human receptor according to the invention, produced by such host cells according to the method well known by the person skilled in the art.

[0027] The present invention is also related to a transgenic non human mammal comprising a partial or total deletion of the genetic sequences encoding the receptor according to the invention, preferably a non human mammal

comprising an homologous recombination "knock-out" of the nucleotide sequences (polynucleotides) according to the invention or a transgenic non human mammal overexpressing above natural level said nucleotide sequences (polynucleotides).

[0028] Said transgenic non human mammal can be obtained by methods well known by the person skilled in the art, for instance by the one described in the document WO98/20112 using classical techniques based upon the transfection of embryonic stem cells, preferably according to the method described by Carmeliet et al., *Nature*, Vol. 380, p. 435-439, 1996.

[0029] Preferably, said transgenic non human mammal overexpressing the polynucleotides according to the invention or portions thereof comprises said polynucleotides or active portions thereof incorporated in a DNA construct with an inducible promoter allowing its overexpression and possibly tissues and other specific regulatory elements.

[0030] Another aspect of the present invention is related to a method for the screening (detection and possibly recovering) of (odorant, pheromones or inhibitor thereof) compounds which are known or not known to be agonists, antagonists or inhibitors of natural compounds to the receptor according to the invention, said method comprising :

- contacting a cell or cell extract from the cell transfected with a vector expressing the polynucleotides encoding the receptor(s) according to the invention or active portion(s) thereof,
- possibly isolating a membrane fraction from the cell extract or the complete cell with a natural compound binding to said receptor under conditions permitting binding of said compound to said receptor, possibly by

the activation of a functional response (resulting in a detectable signal) and

- detecting the presence of any such compound by means of a bioassay, preferably a modification in the production of a second messenger or an increase in the receptor activity in the presence of another molecule (or compound) working as an agonist, antagonist or inhibitor of a natural known compound to the receptor according to the invention and thereby possibly recovering and determining whether said molecule (or other compound) is able to work as an agonist, antagonist or inhibitor of the natural compound to its receptor, preferably the second messenger assay comprises the measurement of intra-cellular cAMP, intracellular inositol phosphates, intra-cellular diacylglycerol concentration, arachinoid acid concentration, MAP kinase(s) or tyrosine kinase(s) pathways or intra-cellular calcium mobilisation.

[0031] The present invention is related to said molecules or compounds, preferably pheromones or flavours, including possible toxic molecules identified by said screening (identification and recovering) method and to their pharmaceutical, cosmetical and industrial (production of detergents, soap, shampoo, fragrances, odorant fingerprints, appetite surpressant compounds, etc.) use.

[0032] Such molecules or compounds may be used also for obtaining in mammal a modification of its taste and its physiological reactions to odorant, pheromones or flavours.

[0033] The present invention is also related to a (preferably nasal) spray for controlling appetite comprising the identified compounds or molecules by the method according to the invention in a suitable carrier.

[0034] Another application of such receptor is the trapping of odour by using the receptor, the cell or membrane according to the invention wherein the desired

odour ligand is absorbed by the binding of the odorant ligand to the odorant receptor.

[0035] The present invention is further related to an odour trap, using said method for trapping odour.

[0036] Preferably, the tested odorants or pheromones upon the receptor according to the invention are advantageously selected from different body secretions such as sweat, salivary, urine, vaginal secretions, sperm, etc. The tested odorants or pheromones upon the receptor are also advantageously selected from the group of 16-androstene family, such as the 5α-androst-16-en-3α-ol, the 5α- androst-16-en-3-one, androstadienone, a human pheromone described previously (Grosser et al., *Psychoneuroendocrinology* vol. 25, pages 289-299, 2000) and other androstenol derivatives; the group consisting of estrene family, such as 1,3,5(10),16-estratetraen-3-ol and other estradiol derivatives described in PCT/US92/00219, PCT/US92/00220 and EP0562843 ; the group consisting of progestin family such as the human pheromone pregna-4,20-diene-3,6-dione (Monti-Bloch et al., J. Steroid Biochem. Mol. Biol. Vol. 65, pages 237-242) and other progesterone derivatives , the group consisting of small fatty acids such as acetic acid, propionic acid, butyric acid, isovaleric acid and isocaproïc acid that compose the putative human vaginal pheromone copulin and such as the trans-3-methyl-2-hexanoïc acid found in human sweat, cyclic organic compounds homologs to known animal pheromone such as dehydro-*exo*-brevicomin and such as nepetalactone, and human homologs of the murin protein aphrodisin.

[0037] Other examples of such compounds are molecules present in vapour emanating from narcotics, like cocaine, marihuana, heroin, hashish, angel dust, gasoline, natural gas, alcohol, decayed human flesh, explosives,

plastic explosives, fire arms, gun powder, toxic fumes, noxious fumes or dangerous fumes, etc.

[0038] Said molecules or compounds could be used also for promoting or suppressing chemical communication. Every stimuli involving the emission by an organism or an exogenous source of chemical compounds able to modify the probability of response of another organism or a part of another organism.

[0039] These molecules or compounds could be used in the treatment or prevention of various disorders affecting, for instance, cell migration, cell death, cell growth, psychotic and neurological disorders, including anxiety, schizophrenia, maniac depression, depression or mound modification, etc.

[0040] These molecules or compounds could be used also for improving contraceptive medication, treatment promoting axonal growth, neural connection and nerves regeneration, in modulating male and female endocrine functions, or may effect the menstrual cycle. Indeed, it is known that several of said receptors can be present at the surface of spermatozoids and can then find contraceptive application or could be used in the treatment/prevention of sterility/fertility. these receptors are also present in various neurolfactory neurons and can improve their connection, especially upon the olfactory bulb or upon other tissue comprising said olfactory receptors.

[0041] They could be also used for the treatment or the prevention of various animal or human behaviours, such as stimulation and/or suppression of appetite, stimulation and/or suppression of motivation and sexual attraction, stimulation and/or suppression of aggressivity, stimulation and/or suppression of alarm and defence behaviours, stimulation and/or suppression of territory and

trail-marking, stimulation and/or suppression of social regulation and recognition, stimulation and/or suppression of mother-child recognition, etc.

[0042] The screening method according to the invention could be performed by well known methods to the person skilled in the art, preferably high-throughput screening, diagnostic and dosage devices based upon the method described in the International patent application WO00/02045 performed upon various solid supports such as micro-titer plates or biochips according to known techniques by the person skilled in the art.

[0043] The present invention is also related to the molecules characterized and possibly recovered by said method, including the pharmaceutical composition comprising a sufficient amount of said molecules and a pharmaceutically acceptable carrier or diluent for the preparation of a medicament in the prevention and/or the treatment of specific diseases.

[0044] A last aspect of the present invention is related to a biosensor or any technical device comprising the receptors according to the invention for the detection of the specific above-mentioned compounds or molecules.

CLAIMS

1. Pheromone G-protein coupled receptor having an amino acid sequence which presents 95% sequence identity with the SEQ ID NO. 1.

2. G-protein coupled receptor having the amino acid sequence SEQ ID NO. 1 or a specific active portion thereof.

3. Polynucleotide encoding any of the amino acid sequences of the G-protein coupled receptor according to claim 1 or 2.

4. Agonist, antagonist or inhibitor of the receptor or the polynucleotide according to any of the preceding claims.

5. Vector comprising the polynucleotide according to the claim 3.

6. Cell transformed by the vector according to the claim 5.

7. Non-human mammal comprising a partial or total deletion of the polynucleotide according to the claim 3 encoding the receptor according claim 1 or 2, preferably an non-human mammal comprising an homologous recombination "knock-out" of said polynucleotide or a transgenic non-human mammal overexpressing above natural level said polynucleotide.

8. Compound selected from the group consisting of agonist, antagonist or inhibitor of the receptor or the polynucleotide according to any of the claims 1 to 3.

9. Method for the screening (detection and possibly recovering) of compounds which are known or not known to be agonists, antagonists or inhibitors of natural compounds to the receptor according to claim 1 or 6, said method comprising :

- contacting a cell or cell extract from the cell transfected with a vector according to the claim 5,
- possibly isolating a membrane fraction from the cell extract or the complete cell with a natural compound binding to said receptor under conditions permitting binding of said compound to said receptor, possibly by the activation of a functional response, and
- detecting the presence of any such compound by means of a bioassay, preferably a modification in the production of a second messenger or an increase in the receptor activity in the presence of another molecule (or compound) working as an agonist, antagonist or inhibitor of a natural known compound to the receptor and thereby recovering and determining whether said molecule (or other compound) is able to work as an agonist, antagonist or inhibitor of the natural compound to its receptor.

10. Compound identified by the screening method according to the claim 9.

11. Pharmaceutical composition comprising an adequate pharmaceutical carrier and a sufficient amount of the receptor according to claim 1 or 2, the poly-nucleotide encoding said receptor according to the claim 3, the agonists, antagonists or inhibitors according to the claim 4 or the vectors according to the claim 5 or the cell according to the claim 6.

12. Use of the agonists, antagonists or inhibitors according to the claim 4 or the compound according to the claim 10 for the improvement, elimination or substitution of an existing taste and/or a fragrance of (or in) food and/or cosmetical products.

13. Use of the agonists, antagonists or inhibitors according to the claim 4 or the compound according to the claim 10 for the preparation of a

medicament in the treatment and/or the prevention of a disorder affecting a mammal, including a human, such as disorders in cell migration, sterility, psychotic and neurological disorders, including anxiety, schizophrenia, maniac depression, depression, for promoting axonal growth, nerve cell connection and nerve regeneration, for modulating male and female endocrine functions, hormone production and menstrual cycle, for the prevention or the treatment by stimulation of several mammal (including human) behaviours, such as stimulation or suppression of appetite, stimulation or suppression of sexual motivation and sexual attraction, stimulation or suppression of aggressivity and for promoting or suppressing chemical communication between organisms.

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